(1984) *Cell (Cambridge, Mass.) 36*, 993–1005.
Luhrmann, R., Appel, B., Bungmann, P., Rinke, J., Reuter, R., Rothe, S., & Bald, R. (1982) *Nucleic Acids Res. 10*, 7103–7113.
Madore, S. J., Wieben, E. D., & Pederson, T. (1984) *J. Cell Biol. 98*, 188–192.
Mattaj, I. W., & DeRobertis, E. M. (1985) *Cell (Cambridge, Mass.) 40*, 111–118.
Pace, B., Stahl, D. A. & Pace, N. R. (1984) *J. Biol. Chem. 259*, 11448–11453.
Pelham, H. R. B., & Jackson, R. J. (1975) *Eur. J. Biochem. 67*, 247–256.
Stahl, D. A., Pace, B., Marsh, T., & Pace, N. R. (1984) *J. Biol. Chem. 259*, 11448–11453.
Stark, B. C., Kole, K., Bowman, E. J., & Altman, S. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3717–3721.
Wieben, E. D., Nenninger, J. M., & Pederson, T. (1985) *J. Mol. Biol. 183*, 69–78.
Yuo, C.-Y., Area, M., & Weiner, A. M. (1985) *Cell (Cambridge, Mass.) 42*, 193–202.

The σ Subunit of RNA Polymerase Contacts the Leading Ends of Transcripts 9–13 Bases Long on the λ P_R Promoter but Not on T7 A1[†]

Susan L. Bernhard and Claude F. Meares*
Department of Chemistry, University of California, Davis, California 95616
Received January 27, 1986; Revised Manuscript Received May 8, 1986

ABSTRACT: The σ subunit of RNA polymerase is responsible for specific initiation of RNA synthesis at promoter sites on DNA. σ dissociates shortly after initiation. Photoaffinity-labeling experiments performed on transcription complexes with two different DNA promoters, which have highly homologous control sequences upstream from the transcribed regions, have revealed that the σ subunit of RNA polymerase is contacted by the 5′ ends of quite different lengths of nascent RNA in each transcription complex. On the other hand, the labeling of subunits $\beta\beta'$ is quite similar for both promoters, and the α subunit is not labeled in either case. The results of transcription experiments on the phage λ P_R promoter show that σ can be photoaffinity labeled by RNA chains that are 9–13 nucleotides long and thus remains associated with the core enzyme at least to that point. But on the A1 promoter of phage T7 DNA, photoaffinity labeling of σ ceases with the trinucleotide. Thus release of σ from the vicinity of nascent RNA depends not merely on the length but on the sequence of the transcript. For the T7 A1 promoter, σ labeling ceases while the leading end of the RNA is still base paired to the DNA template; thus, it appears that there is at least one site on the enzyme that interacts with the growing transcript/template hybrid, in a sequence-dependent way, to effect σ release. Similarities with the results of Hansen and McClure [Hansen, U. M., & McClure, W. R. (1980) *J. Biol. Chem. 255*, 9564–9570] for σ dissociation from transcription complexes on poly[d(A-T)] are discussed, with the observation that the RNA/DNA hybrids formed on T7 A1 show an alternating pattern of bridging hydrogen bond donors and acceptors in the major groove that exactly matches the one formed on poly[d(A-T)]. This property is not shared by λ P_R.

DNA-dependent RNA polymerases catalyze the synthesis of ribonucleic acid, with deoxyribonucleic acid as a template (Losick & Chamberlin, 1976; von Hippel et al., 1984; McClure, 1985; Doi, 1977). These enzymes are usually large, multisubunit assemblies; *Escherichia coli* RNA polymerase contains five major subunits, with a total molecular weight of 449K. The "core" enzyme consists of subunits β' (M_r 155 162; Ovchinnikov et al., 1982) and β (M_r 150 619; Ovchinnikov et al., 1981), and two α subunits (M_r 36 512; Ovchinnikov et al., 1977); the core enzyme is capable of elongating RNA but does not specifically initiate transcription at promoter sites on DNA. The RNA polymerase holoenzyme contains the core plus the dissociable subunit σ (M_r 70 263; Burton et al., 1981), which is required for specific initiation of transcription.

Since the discovery of the σ subunit (Burgess et al., 1969) and its ability to act catalytically in the initiation of transcription (Travers & Burgess, 1969), the mechanism by which σ acts has interested many investigators (Chamberlin, 1974; Hansen & McClure, 1980; von Hippel et al., 1984; McClure, 1985). It is frequently assumed that σ dissociates from the transcription complex shortly after formation of the first phosphodiester bond (Lewin, 1983), but the elegant experiment of Hansen and McClure (1980) indicates that σ remains bound until either eight or nine bases have been transcribed on a poly[d(A-T)] template. No other σ dissociation experiments have been described in the literature, presumably because they are very difficult technically, but it has been emphasized recently that the question of when and why σ dissociates from a transcription complex is of fundamental importance for understanding the mechanism of transcription (von Hippel et al., 1984; McClure, 1985).

Photoaffinity experiments with RNA analogues in transcription complexes can provide relevant information, since if photoaffinity labeling of σ occurs, it must have been part of a transcription complex. DeRiemer and Meares (1981b) found that a 5′-azide photoprobe on a di- or trinucleotide

[†]Supported by Research Grant GM 25909 to C.F.M. from the National Institute of General Medical Sciences, NIH.

0006-2960/86/0425-5914$01.50/0

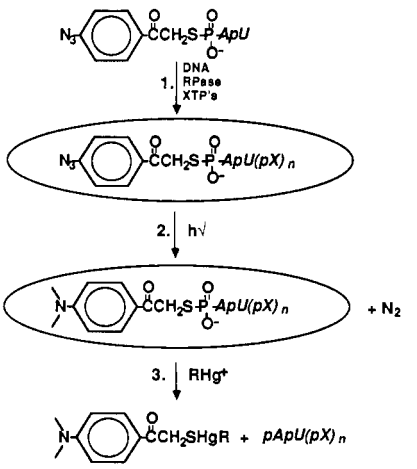

FIGURE 1: Chemical reactions involved in experiments described here. (1) Synthesis of RNA from 5′-azide dinucleotide initiator in a transcription complex. (2) Irradiation of the transcription complex leading to insertion of N into a chemical bond on the enzyme. (3) Cleavage of RNA from labeled enzyme subunit performed after separation from the other subunits.

labeled the σ subunit in poly[d(A-T)] transcription complexes but that elongation to a tetranucleotide decreased the labeling markedly. Hanna and Meares (1983a) found that a 5′-azide on a trinucleotide labeled σ in a transcription complex on the T7 A1 promoter, but neither a tetranucleotide nor any longer nucleotide did so (Hanna & Meares, 1983b).

For some time we have been interested in the entire path that nascent RNA follows through the transcription complex (DeRiemer & Meares, 1981a,b; Hanna & Meares, 1983a,b). Using photoaffinity-labeling methods, with a light-activated aryl azide positioned at the leading (5′) end of nascent RNA, we have mapped that path at the level of resolution of the enzyme subunits and DNA using the A1 promoter of bacteriophage T7 deletion mutants ΔD111 and ΔD123 (Hanna & Meares, 1983a,b). When the RNA is 3–12 bases long, the DNA template receives most of the photoaffinity labeling: for trinucleotide RNA, the β and σ subunits are just detectably labeled but not β′ or α; for 4–12 nucleotide RNA, the β and β′ subunits are detectably labeled but not α or σ. For RNA longer than 12 nucleotides (and as long as 94 nucleotides on T7 ΔD123), the β and β′ subunits are very heavily labeled, and no significant labeling of the other macromolecules occurs (not even for RNAs as long as 116 nucleotides).

These results contrast with an earlier, less complete study by Grachev and Zaychikov (1981). Those authors found that, in transcription complexes on a phage T7 DNA fragment containing four promoters (A0, A1, A2, and A3), a 5′-azide photoprobe on a di- or hexanucleotide labeled σ significantly, and a di-, hexa-, or dodecanucleotide labeled β. An important question raised by comparing both sets of results is whether the path of nascent RNA is affected by the nature of the DNA template or whether different natural promoter sites on DNA will force the enzyme into different conformations or change the properties of the transcription complex in some other way.

Hanna and Meares (1983a,b) used the cleavable dinucleotide photoaffinity probe shown in Figure 1, which (1) can serve as a specific RNA chain initiator on promoters whose transcripts begin with A-U, (2) is converted to a highly reactive nitrene by irradiation with UV light, and (3) after isolation of photoaffinity-labeled products, can be cleaved to release the intact RNA chain responsible for the labeling. This probe can be used with either T7 A1 or the λ P_R promoter. Here we compare photoaffinity experiments of those two promoters with the remarkable finding that, on the P_R promoter of phage λ, 5′-azide photoprobes on RNAs from 9 to 13 bases long readily photoaffinity label σ.

Experimental Procedures

Materials

All reagents and solvents were the purest available and were used without further purification unless noted otherwise. Deionized, glass-distilled water was used throughout. *E. coli* MRE 600 cells were purchased from Grain Processing Corp. Bactotryptone and yeast extract were from Difco. Ribonucleoside triphosphates, purchased from Sigma, were twice column purified (McClure et al., 1978) before use. Soluble RNA, phenylmercuric acetate, DTT,[1] tetramethylethylenediamine, bis(acrylamide), cordycepin triphosphate (3′-deoxy-ATP, a chain-terminating ATP analogue), agar, Tris, acrylamide, poly(ethylene glycol) (M_r 6000), ethidium bromide, heparin, and cesium chloride were purchased from Sigma. *p*-Azidophenacyl bromide was from Pierce, adenosine 5′-*O*-thiophosphate was from Boehringer-Mannheim, and 3′-*O*-methyl-CTP, -GTP, and -UTP (RNA chain terminators) were from P-L Biochemicals. α-^{32}P-Labeled nucleoside triphosphates (410 Ci/mmol) were purchased from Amersham. *Hin*dIII restriction endonuclease was purchased from Bethesda Research Laboratories, and *Eco*RI was from New England Biolabs. Kodak XAR film and Cronex Lightning-Plus intensifying screens were used for autoradiography.

Buffers. These were as follows: buffer A contained 80 mM Tris-HCl, pH 7.9, 5 mM 2-mercaptoethanol, 0.1 mM Na_2-EDTA, and 50% glycerol; buffer B contained 10 mM Tris-HCl, pH 8.0, and 1 mM Na_2EDTA; buffer C contained 50 mM Tris-HCl, pH 7.9, 5 mM 2-mercaptoethanol, 10 mM NaCl, 10 mM $MgCl_2$, and 5% (v/v) glycerol; buffer D contained 0.5 mL of buffer F, 2.9 mL of 9–10 M urea, 0.5 g of sucrose, 12 mg of $NaDodSO_4$, 0.1 mL of 0.1% (w/v) bromphenol blue, and 10 mM dithiothreitol (added just before use); buffer E contained, in 1 L, 4.4 mL of ethanolamine, 4.5 g of glycine, and 1.0 g of $NaDodSO_4$ (pH 9.7); buffer F contained 18.6 mL of triethanolamine, 8 mL of concentrated HCl, and 96 g of urea in 200-mL total volume, pH 7.5; buffer G (BRL core buffer) contained 50 mM NaCl, 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, and 100 μg/mL bovine serum albumin (added just before use).

Cleavage Solution. First, 0.1% (w/v) $NaDodSO_4$ is saturated with phenylmercuric acetate by shaking with an excess of the pure solid for a minimum of 4 h at room temperature. Then the mixture is briefly centrifuged to settle the precipitate, and the precipitate is discarded. Just before use, 1 mg/mL soluble RNA is added as a carrier.

Methods

RNA polymerase was purified from *E. coli* MRE 600 cells according to the method of Burgess and Jendrisak (1975) as modified by Lowe et al. (1979). The enzyme was dialyzed against buffer A and stored at −20 °C. Protein concentrations were determined by the methods of Bradford (1976) and Sedmak and Grossberg (1977).

DNA Templates. Bacteriophage T7 ΔD111 stock and the *E. coli* C_1A host strain were kindly provided by Judith Levin and Michael Chamberlin. The phage was grown and harvested essentially according to the method of Yamamoto et al. (1970).

[1] Abbreviations: DTT, dithiothreitol; ATP, adenosine 5′-triphosphate; Tris, tris(hydroxymethyl)aminomethane; CTP, cytidine 5′-triphosphate; GTP, guanosine 5′-triphosphate; UTP, uridine 5′-triphosphate; EDTA, ethylenediaminetetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate; HPLC, high-performance liquid chromatography; NTP, unspecified nucleoside 5′-triphosphate.

The phage particles were concentrated with poly(ethylene glycol) and banded in a CsCl step gradient in a Beckman L2-65B ultracentrifuge (Maniatis et al., 1982). After isolation, phage particles were disrupted with $KDodSO_4$ (Schleif & Wensink, 1981); the partially purified DNA was extracted with phenol, dialyzed against buffer B, and stored at 4 °C.

Bacteriophage λ P_R promoter was obtained from plasmid pGW7, prepared by Geoff Wilson and William Konigsberg and kindly provided by Martin Schmidt and Michael Chamberlin. It was grown in an *E. coli* JM83 host (kindly provided by Ikuhisa Sawada and Carl Schmid) at 28 °C without amplification and isolated by using alkali lysis and a CsCl density gradient (Maniatis et al., 1982). The isolated plasmid was digested with *Eco*RI and *Hin*dIII, and the 933 base pair fragment containing the λ P_R promoter was isolated by electrophoresis in 0.8% agarose (Maniatis et al., 1982). The ends of this fragment correspond to positions 37584 and 39168 in the λ genome (Sanger et al., 1982) (positions 38103–38754 deleted). DNA concentration was determined by measuring absorbance at 260 nm (one A_{260} unit = 50 μg/mL).

Dinucleotide Photoaffinity Probe. 5′-[(4-Azidophenacyl)thiophosphoryl]adenylyl(3′–5′)uridine (Figure 1) was synthesized as reported by Hanna and Meares (1983a). An abortive initiation reaction catalyzed by RNA polymerase on a poly[d(A-T)] template, using adenosine 5′-*O*-thiophosphate and UTP as substrates, produced the dinucleotide 5′-*O*-thiophosphoryladenylyl(3′–5′)uridine, which was isolated by HPLC. Alkylation of the dinucleotide by *p*-azidophenacyl bromide was carried out in reduced light, and excess *p*-azidophenacyl bromide was extracted with isobutyl alcohol, followed by ethyl ether. The photoprobe was isolated by HPLC. Product concentration was determined by using $\epsilon_{300} = 2 \times 10^4\ M^{-1}\ cm^{-1}$.

Preparation for Transcription Experiments. A total of 12 different transcription reactions were run in a single experiment to give a distribution of RNA chain lengths (3- to 80-mers). For each of four sets of reactions, one base-specific RNA chain terminator was used at three concentrations to generate short, medium, and long RNA transcripts.

First, two master mixes were prepared: an initiating NTP mix and an elongating NTP mix.

The initiating NTP mix was prepared from unlabeled ATP, CTP, GTP, UTP, and [α-^{32}P]GTP (except that UTP was omitted here for T7 A1). Once prepared and thoroughly mixed, this was aliquoted into 12 vials and stored on ice. Concentrations of the initiating NTPs were chosen so that they would be 1 μM for ATP and 3 μM for CTP, UTP, and GTP, after addition to an aliquot of the ternary complex mixture (described below).

The elongating NTP mix was composed of unlabeled ATP, CTP, GTP, and UTP. Twelve aliquots (three for each of the four base-specific terminators) were put into separate vials. The amount of substrate was chosen so that each reaction would contain each NTP at 15 μM in a final reaction volume of 80–90 μL. The resulting fivefold dilution of the [α-^{32}P]GTP served to limit the increase in specific radioactivity with chain length.

Base-specific terminators were added to aliquots of the elongating NTP mix in the following manner. For the T7 A1 experiments, short RNA chain lengths (out to 20-mers) were prepared by using terminator-to-NTP ratios of 125/1 for ATP, 100/1 for CTP, and 50/1 for GTP; medium RNA chain lengths (21- to ~60-mers) were prepared by using ratios of 45/1 for ATP and CTP, 20/1 for GTP, and 40/1 for UTP; longer RNA chains were made with 10/1 ratios for ATP, CTP, and UTP and 5/1 for GTP. For the λ P_R experiments, short RNA chain lengths were prepared by using terminator-to-NTP ratios of 75/1 for ATP, 50/1 for CTP, 25/1 for GTP, and 30/1 for UTP; medium lengths were prepared by using ratios of 60/1 for ATP, 50/1 for CTP, 25/1 for GTP, and 30/1 for UTP; long chains were prepared by using 5/1 for ATP or GTP and 10/1 for CTP or UTP.

Detailed Description of Transcription Experiments. All transcription reactions were carried out in dim light (15-W incandescent bulb). For proper timing, the three A-terminated reactions were carried to completion (including irradiation), then the C-terminated reactions, and so on.

A ternary complex mix was prepared, containing 15 nM promoter, 100 nM *E. coli* RNA polymerase, and 100 μM 5′-[(4-azidophenacyl)thiophosphoryl]adenylyl(3′–5′)uridine, in buffer C. Once prepared and thoroughly mixed, this was aliquoted into four vials, one for each set of base-specific terminator reactions, and stored on ice in reduced light until needed. For each terminator, ternary complex mixtures were incubated for 10 min at 37 °C in reduced light. At the end of this time, the ternary complex was aliquoted into three vials containing initiating NTPs; then elongating NTPs (premixed with terminator) were added to individual vials at 0.5, 1.0, and 1.5 min to generate short, medium, and long RNA chains. Elongation was allowed to proceed for approximately 10 min at 37 °C. At the end of the elongation period, a 5-μL aliquot was removed from each reaction; this sample was run on an RNA sequencing gel to confirm the lengths of RNA made. Then the three reaction mixtures for a given terminator were pooled. In our hands, the combination of three separate elongation reactions in this manner produced the best overall distribution of RNA lengths (reaction 1 in Figure 1).

An aliquot was removed for an unirradiated control sample, and the remainder of the sample was transferred to a borosilicate tube and irradiated for 1 min in a Rayonet photochemical reactor (λ > 300 nm). Following irradiation (reaction 2 in Figure 1), each sample was denatured by addition of an equal volume of buffer D and allowed to stand in the dark at room temperature for 1 h. Samples then were frozen at −20 °C or loaded directly onto a protein gel.

Protein Gels. The protein gel used in these experiments was the $NaDodSO_4$–urea system of Wu and Bruening (1971). The gel was 7% polacrylamide for the T7 A1 experiments and 5% for λ P_R. These preparative gels separated the photoaffinity-labeled σ subunit of RNA polymerase from the DNA template and the other polymerase subunits. Gels were run for 8–10 h at 10–15 mA or until the bromphenol blue tracking dye reached the bottom of the gel.

After electrophoresis the gels were wrapped in plastic wrap and autoradiographed at −79 °C with Kodak XAR film and an intensifying screen. Usually, a 12-h minimum exposure time was required to visualize the subunits.

Electroelution and Cleavage. Photoaffinity-labeled σ or ββ′ subunits, located by autoradiography and by comparison with stained marker lanes, were excised from the protein gels and transferred to 1.5-mL polypropylene Eppendorf centrifuge vials. Gel slices in vials were counted (Cerenkov radiation) in a Beckman LS 6800 scintillation counter prior to electroelution.

Electroelution was carried out by placing individual gel slices plus 200 μL of buffer E into pieces of dialysis tubing. The dialysis bags were placed in a tray containing buffer E, and electroelution was performed at 50 mA, 4 °C, for 4–6 h. This procedure eluted roughly 40–50% of the counts from protein gel slices.

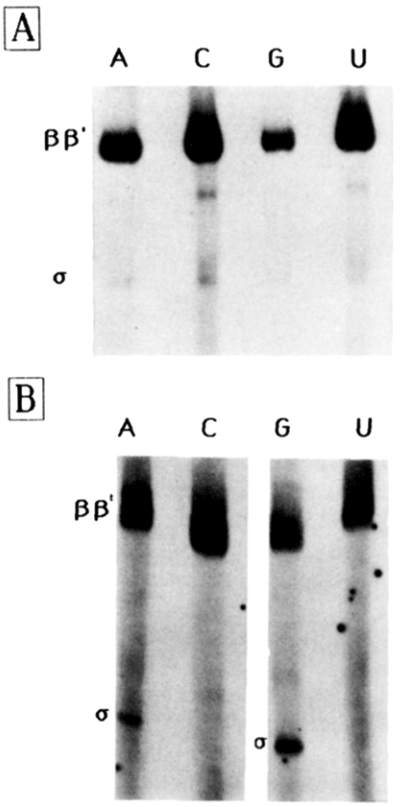

FIGURE 2: Autoradiograms of $NaDodSO_4$–urea polyacrylamide gels, comparing the extent of σ labeling on two different promoters. Labels A, C, G, and U indicate that each reaction mixture contained a chain-terminating analogue of one of those nucleotides. On the T7 A1 promoter (panel A) σ labeling is negligible. Significant labeling is observed on the λ P_R promoter (panel B) in the reactions terminated with A or G analogues. RNA transcripts 9–13 nucleotides long on λ P_R end with one of these bases.

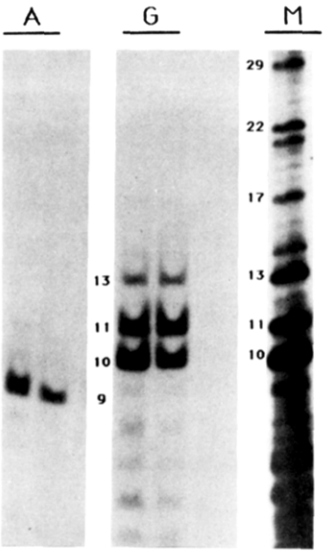

FIGURE 3: Autoradiogram of an RNA sequencing gel, showing the distribution of RNA chain lengths that label the σ subunit on the λ P_R promoter. σ is labeled by RNA chains that are 9–13 nucleotides long, even though considerable quantities of longer and shorter nucleotides are present. Lane A shows (in duplicate) that the 9-mer terminating in A was attached to σ; lane G shows that the 10-, 11-, and 13-mers terminating in G were attached to σ. Lane M shows all the transcripts present in the G-terminated reaction mixture. The 12-mer terminating in A was present in relatively low abundance in the A reaction mixture; this may explain the absence of a 12-mer band in lane A.

The eluted protein–RNA conjugates were transferred to Eppendorf vials. Each dialysis bag was rinsed with 200 μL of freshly prepared cleavage solution. The rinse was pooled with the original eluant; cleavage (reaction 3 in Figure 1) was allowed to proceed for 18–24 h at room temperature in the dark.

Following the cleavage reaction samples were lyophilized (8 h, 20–50 mtorr) and then resuspended in 15 μL of 8 M urea (2 h at room temperature). Marker dyes were added [0.05% (w/v) bromphenol blue and xylene cyanol] and 10-μL aliquots were loaded onto RNA sequencing gels.

RNA Sequencing Gels. These were 40 cm × 15 cm × 0.75 mm 25% acrylamide gels, containing 89 mM Tris–borate (pH 8.3), 1 mM EDTA, 7 M urea, and 1:29 methylenebis(acrylamide):acrylamide (Carpousis & Gralla, 1980). Gels were allowed to polymerize overnight and were preelectrophoresed for 6 h at 1000 V before use. For analysis of RNA chain lengths, gels were run at 1000 V for 12 h or until the bromophenol tracking dye was 27.5 cm from the bottom of the wells. After electrophoresis, gels were placed between layers of plastic wrap and autoradiographed with intensifying screens at −79 °C.

RESULTS

Figure 2 compares typical autoradiograms of protein gels observed after photoaffinity experiments on the T7 A1 promoter (panel A) or the λ P_R promoter (panel B). Note in particular that the σ subunit is not significantly labeled in any of the T7 A1 lanes (terminated by A, C, G, or U analogues); almost all the RNAs in these experiments were four or more nucleotides long and thus did not label σ. However, σ is clearly labeled in the A- and G-terminated lanes for the λ P_R experiments; the λ P_R transcript ends in either A or G when it is 9–13 bases long. No labeled σ appears in the C- or U-terminated lanes for the λ P_R experiments. The gels in Figure 2 are typical results from experiments that were repeated several times. For the gel shown in panel B for λ P_R, the labeled bands in the A lane contained 1364 cpm for ββ′ and 411 cpm for σ; the G lane contained 1032 cpm for ββ′ and 543 cpm for σ. For the T7 A1 experiments, labeling of σ was <5% of ββ′ labeling.

Figure 3 shows the lengths of RNA transcripts whose 5′ ends were photoattached to σ in the λ P_R experiments. It also shows the relative amounts of all RNAs that were present in the G-terminated reaction mixture; reactions terminated with A, C, or U analogues also contained a variety of chain lengths. In all cases, RNA lengths shorter than nine nucleotides were present in abundance yet did not label σ significantly. Labeling of σ by RNAs from 9 to 13 nucleotides long is readily observable; the relatively large photoaffinity-labeling yield on σ clearly indicates that the 5′ end of the transcript is closely associated with σ when the transcript is 9–13 bases long. Heavy labeling of ββ′ by RNAs from 13 to >80 nucleotides long was also readily observed on λ P_R (this is quite similar to T7 A1); no labeling of the α subunits was detected with either DNA template. The λ P_R DNA was not analyzed for photoaffinity labeling.

DISCUSSION

It is evident that the σ subunit does not dissociate from the λ P_R transcription complex unitl after the transcript has reached a length of 13 nucleotides. It is also evident that, on λ P_R, azides on the (abundantly present) RNAs that are 4–8 nucleotides long do not label σ. On poly[d(A-T)] or T7 A1 DNA, photoaffinity labeling by trinucleotides is observable only under conditions that strongly favor formation of trinucleotides rather than longer transcripts (DeRiemer &

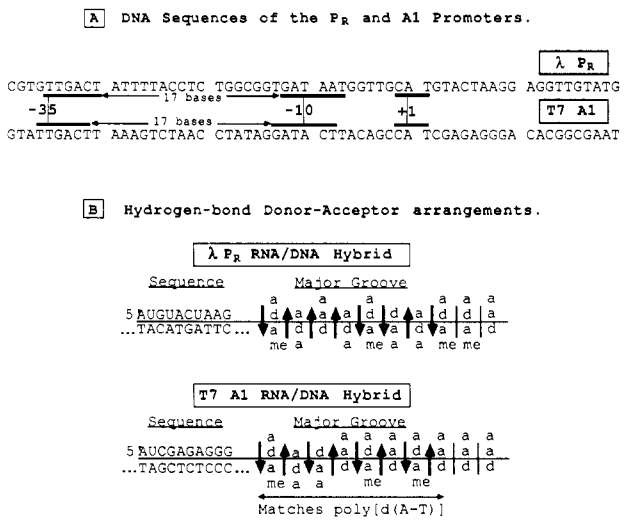

FIGURE 4: (A) Comparison of the sequences of the λ P_R and T7 A1 promoters, showing the extensive homology of the upstream regions. (B) Comparison of the spatial distributions of bridging hydrogen bond donors and acceptors in the major groove of the transcript/template hybrid formed at each promoter (von Hippel, 1979). Arrows point from donor (d) to acceptor (a), and homology with the poly[d(A-T)] hybrid is indicated at bottom. The C-5 methyl (me) of thymine is also shown.

Meares, 1981b; Hanna & Meares, 1983a). Such conditions were not investigated for λ P_R DNA.

The observed labeling by lengths 9–13 is consistent with the possibility that σ is bound near one side of the growing RNA–DNA helix in the transcription complex and that the labeling occurs as the 5′ end of nascent RNA passes across this side. (Because the 3′ end of the RNA must remain at the active site of the enzyme, the 5′ end must translocate with respect to the enzyme as new bases are added.)

A markedly different result is obtained with the T7 A1 promoter. On that promoter, the 5′-azide probe labels DNA, β, and β′ when the RNA is 9–13 bases long but does not label σ (Hanna & Meares, 1983b). The simplest explanation is that σ has dissociated from the A1 transcription complex before the RNA has reached a length of nine nucleotides. This is supported by the observation that labeling of the other enzyme subunits is similar with both promoters.

There is another possibility that σ has not dissociated but rather is in a different position relative to the transcript in the T7 A1 transcription complex so that it is inaccessible to the photoaffinity probe. We favor the first explanation, because (1) on T7 A1, probes with lengths 9–13 label three different macromolecules (β, β′, and DNA) and thus appear to be free to label any molecule in their immediate surroundings, and (2) since lengths 9–13 do label σ on λ P_R, it would be necessary to postulate that either σ or the transcript/template hybrid has more than one binding site on the transcription complex in order to explain the T7 A1 results.

Why should σ dissociate from different transcription complexes at different points in RNA synthesis? Since it is not simply an effect of transcript length, it appears that the signal for retention or dissociation of σ is provided by the sequence of the nucleic acids involved. Figure 4A shows that, for both the λ P_R and T7 and A1 promoters, the upstream control sequences (von Hippel et al., 1984; McClure, 1985) near positions −10 and −35, and the spacer between them, are nearly identical. But the *transcribed* regions have little or no homology after the first two bases.

The interaction responsible for σ dissociation on the T7 A1 promoter evidently occurs before the transcript is nine bases long, while the 5′ end of RNA is still hybridized to the DNA template (Hanna & Meares, 1983b). Therefore, the signal is likely to be found in the RNA–DNA hybrid.

It has been suggested previously that interactions between the enzyme and GC-rich regions of the nascent RNA/DNA hybrid play a role in other properties of the transcription complex, such as pausing or terminating transcription (Kassavetis & Chamberlin, 1981; Kingston et al., 1981; Reisbig & Hearst, 1981; Morgan et al., 1983; Platt, 1981). However, the sequences in Figure 4A show that there is little difference in GC content in the first few positions of the two transcripts considered here.

It has also been pointed out by several authors that specific interactions between proteins and double-stranded nucleic acids can depend on the spatial distribution of hydrogen bond donors and acceptors in the major groove of the nucleic acid duplex (von Hippel et al., 1982; von Hippel, 1979; Seeman et al., 1976; Takeda et al., 1983). Presumably this donor–acceptor distribution exerts its effect by attracting or repelling a matrix of hydrogen bond donors and acceptors on the protein. Some properties of the −10 and −35 promoter control regions may be explained by considering only the bridging donor–acceptor pairs that occur at the interface between the two strands (von Hippel et al., 1982). As shown in Figure 4B, the T7 A1 transcript/template hybrid produces a distribution of donors and acceptors that is very different from the λ P_R hybrid. In fact, the first eight base pairs of the T7 A1 hybrid form a perfectly alternating pattern of bridging donors and acceptors in the major groove. An identical alternating pattern of bridging hydrogen bond donors and acceptors in the major groove of the nascent RNA–DNA hydbrid is also obtained with poly[d(A-T)], for which it is known that σ dissociates when the transcript is either eight or nine bases long (Hansen & McClure, 1980). Thus T7 A1 and poly[d(A-T)] share the properties of early σ release and alternating donor–acceptor patterns. λ P_R is different in both ways; it does not release σ early nor does it possess an alternating donor–acceptor pattern.

Differences in the timing of σ release from T7 A1 and λ P_R may be related to differences in transcriptional regulation on the two templates. Compared to T7 phage, λ has a much more complex system for regulation of its gene expression (Lewin, 1978). The T7 early region that follows the A1 promoter is thought to be free of transcriptional controls. The cro gene, which directly follows the P_R promoter, contains recognition sequences for a number of protein factors (among them the N gene product, nus A, and ρ), which act in conjunction with RNA polymerase to effect a variety of specific transcriptional controls. In particular, a σ-nus A cycle has been described by Greenblatt and Li (1981); successive binding of these factors to core RNA polymerase allows for termination and reinitiation of transcription. Thus, the delayed release of σ from transcription complexes on the λ P_R template may play a role in regulation of transcription since it would limit the onset of nus A binding.

Our results do not provide a direct answer concerning the site on the enzyme that interacts with the transcript/template hybrid to influence σ release. But the λ P_R experiments show that there is a site on σ that can interact with the nascent 9–13-base RNA. There are several possible mechanisms by which the interactions leading to σ retention or dissociation might occur. The arguments presented here support the hypothesis that the pattern of hydrogen bond donors and acceptors in the major groove of the RNA/DNA hybrid is important in this process. This may be further tested by con-

struction of a suitable set of DNA templates containing different early transcript sequences.

ACKNOWLEDGMENTS

We thank Michelle Hanna, Ikuhisa Sawada, Michael Chamberlin, Judith Levin, Martin Schmidt, William Konigsberg, Thomas Stackhouse, William McClure, and Michael McCall for gifts of material and/or helpful discussions about many aspects of this project, and we thank Gloria Marquez for excellent secretarial assistance.

REFERENCES

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.
Burgess, R. R., & Jendrisak, J. J. (1975) *Biochemistry 14*, 4634–4636.
Burgess, R. R., Travers, A. A., Dunn, J. J., & Bautz, E. K. F. (1969) *Nature (London) 221*, 43–46.
Burton, Z., Burgess, R. R., Lin, J., Moore, D., Holder, S., & Gross, C. A. (1981) *Nucleic Acids Res. 9*, 2889–2903.
Carpousis, A. J., & Gralla, J. D. (1980) *Biochemistry 19*, 3245–3253.
Chamberlin, M. J. (1974) *Annu. Rev. Biochem. 43*, 721–775.
DeRiemer, L. H., & Meares, C. F. (1981a) *Biochemistry 20*, 1606–1612.
DeRiemer, L. H., & Meares, C. F. (1981b) *Biochemistry 20*, 1612–1617.
Doi, R. H. (1977) *Bacteriol. Rev. 41*, 568–594.
Grachev, M. A., & Zaychikov, F. R. (1981) *FEBS Lett. 130*, 23–26.
Greenblatt, J., & Li, J. (1981) *Cell (Cambridge, Mass.) 24*, 421–428.
Hanna, M. M., & Meares, C. F. (1983a) *Biochemistry 22*, 3546–3551.
Hanna, M. M., & Meares, C. F. (1983b) *Proc. Natl. Acad. Sci. U.S.A. 80*, 4238–4242.
Hansen, U. M., & McClure, W. R. (1980) *J. Biol. Chem. 255*, 9564–9570.
Kassavetis, G. A., & Chamberlin M. J. (1981) *J. Biol. Chem. 256*, 2777–2786.
Kingston, R. E., Nierman, W. C., & Chamberlin, M. J. (1981) *J. Biol. Chem. 256*, 2787–2797.
Lewin, B. (1978) *Gene Expression*, Vol. 3, Chapters 4 and 7, Wiley, New York.
Lewin, B. (1983) *Genes*, Chapter 10, Wiley, New York.
Losick, R., & Chamberlin, M., Eds. (1976) *RNA Polymerase*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Lowe, P. A., Hager, E. A., & Burgess, R. R. (1979) *Biochemistry 18*, 1344–1352.
Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
McClure, W. R. (1985) *Annu. Rev. Biochem. 54*, 171–204.
McClure, W. R., Cech, C. L., & Johnston, D. E. (1978) *J. Biol. Chem. 253*, 8941–8948.
Morgan, W. D., Bear, D. G., & von Hippel, P. H. (1983) *J. Biol. Chem. 258*, 9565–9574.
Ovchinnikov, Yu. A., Lipkin, V. M., Modyanov, N. N., Chertov, O. Yu., & Smirnov, Yu. V. (1977) *FEBS Lett. 76*, 108–111.
Ovchinnikov, Yu. A., Monastyrskaya, G. S., Gubanov, V. V., Guryev, S. O., Chertov, O. Yu., Modyanov, N. N., Grinkevich, V. A., Makarova, I. A., Marchenko, T. V., Polovnikova, I. N., Lipkin, V. M., & Sverdlov, E. D. (1981) *Eur. J. Biochem. 116*, 621–629.
Ovchinnikov, Yu. A., Monastyrskaya, G. S., Gubanov, V. V., Guryev, S. O., Salomatina, I. S., Shuvaeva, T. M., Lipkin, V. M., & Sverdlov, E. D. (1982) *Nucleic. Acids Res. 10*, 4035–4044.
Platt, T. (1981) *Cell (Cambridge, Mass.) 24*, 10–23.
Reisbig, R. R., & Hearst, J. E. (1981) *Biochemistry 20*, 1907–1918.
Sanger, F., Coulson, A. R., Hong, G. F., Hill, D. F., & Petersen, G. B. (1982) *J. Mol. Biol. 162*, 729–773.
Schleif, R. F., & Wensink, P. C. (1981) *Practical Methods in Molecular Biology*, pp 100–101, Springer-Verlag, New York.
Sedmak, J. J., & Grossberg, S. E. (1977) *Anal. Biochem. 79*, 544–552.
Seeman, N. C., Rosenberg, J. M., & Rich, A. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 804–808.
Takeda, Y., Ohlendorf, D. H., Anderson, W. R., & Matthews, B. W. (1983) *Science (Washington, D.C.) 221*, 1020–1026.
Travers, A. A., & Burgess, R. R. (1969) *Nature (London) 222*, 537–540.
von Hippel, P. H. (1979) in *Biological Regulation and Development* (Goldberger, R. F., Ed.) Vol. 1, pp 279–347, Plenum, New York.
von Hippel, P. H., Bear, D. G., Winter, R. B., & Berg, O. G. (1982) in *Promoters: Structure and Function* (Rodriquez, R. L., & Chamberlin, M. J., Eds.) pp 3–33, Praeger, New York.
von Hippel, P. H., Bear, D. G., Morgan, W. D., & McSwiggen, J. A. (1984) *Annu. Rev. Biochem. 53*, 389–446.
Wu, G. J., & Bruening, G. E. (1971) *Virology 46*, 596–612.
Yamamoto, K. R., Alberts, B. M., Benzinger, R., Lawhorne, L., & Treiber, G. (1970) *Virology 40*, 734–744.